Evidence of functional deficits at the single muscle fiber level in experimentally-induced renal insufficiency

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ABSTRACT

Chronic kidney disease patients present with metabolic and functional muscle abnormalities, called uremic myopathy, whose mechanisms have not yet been fully elucidated. We investigated whether chronic renal insufficiency (CRI) affects skeletal muscle contractile properties at the cellular level. CRI was induced surgically in New Zealand rabbits (UREM), with sham-operation for controls (CON) and samples were collected at 3-months post-surgery, following euthanasia. All protocols had University Ethics approval following national and European guidelines. Sample treatments and evaluations were blinded. Maximal isometric force was assessed in 382 permeabilized psoas fibers (CON, n=142, UREM, n=240) initially at pH7, 10^{o}C ('standard' conditions), in subsets of fibers in acidic conditions (pH6.2, 10^{o}C) and at near physiological temperature (pH7, 30^{o}C and pH6.2, 30^{o}C). CRI resulted in significant smaller average CSA (~11%) for UREM muscle fibers (vs CON, $P<0.01$). At standard conditions, UREM fibers produced lower absolute and specific forces (i.e. force corrected per fiber CSA) (vs CON, $P<0.01$); force increased in 30^{o}C for both groups ($P<0.01$), but the disparity in force remained significantly different. Acidosis significantly reduced force (vs pH7, 10^{o}C $P<0.01$), similarly in both groups (UREM -48% vs CON -43%, $P>0.05$). CRI induced significant impairments in single psoas muscle fibers force generation, only partially explained by atrophy. For the first time we give evidence that CRI affects muscle mechanics at the cellular level.

Keywords: Chronic kidney disease, isometric tension, skinned fibers, specific force, animal model, uremic myopathy

Introduction

Chronic renal insufficiency (CRI) is a major global health problem expected to affect 40-50% of EU and USA populations (Grams et al., 2013; Zoccali et al., 2010) as well millions in Asia (Abraham et al., 2016) and Latin America (Cusumano and González Bedat, 2008). Skeletal muscle is heavily compromised by CRI (Kaltsatou et al., 2015; Sakkas et al., 2003a, 2003b) with patients presenting muscle atrophy, muscle weakness, limited endurance and fatigue intolerance (Campistol, 2002), only partly explained by inactivity (Sakkas et al., 2003b). Collectively described as *uremic myopathy* (Campistol, 2002), this muscular dysfunction spectrum contributes to patients' high cardiovascular and metabolic morbidity and mortality (Johansen et al., 2007; Pereira et al., 2015) and inhibits the efficacy of rehabilitative interventions.

Muscle atrophy occurs in various clinical conditions but also in healthy individuals under sedentary/unloading or weightless conditions (Riley, 2005). It has been linked to smaller cross sectional areas (CSAs) of individual fibers which can cause an overall altered contractile performance (Riley, 2005). In a recent non-surgical CRI animal model, extensor digitorum longus and soleus muscle dysfunction was attributed to atrophy (Organ et al., 2016). However, in another surgical CRI animal model no atrophy was observed in tibialis cranialis nor in soleus muscle (Acevedo et al., 2015). Additional factors such as neuropathy, mitochondrial dysfunction and substrate availability are also implicated [for a review refer to (Adams and Vaziri, 2006)], meaning that atrophy alone may not fully account for the observed muscle dysfunction in CRI. Moreover it is not clear if basic contractile properties are affected.

Fatigue intolerance in CKD has been also attributed to the development of rapid acidosis (low intracellular pH) (Johansen et al., 2005; Moore et al., 1993). It is well known that low intracellular pH inhibits muscle contraction at the sarcomeric protein level due to an effect of H^+ both on the interaction between the motor proteins and on calcium handling [e.g. (Allen et al., 2008; Fabiato and Fabiato, 1978; Karatzaferi et al., 2008; Nelson and Fitts, 2014)]. However, studies employing near physiological temperatures during the functional assessment of isolated fibers have indicated that the force depressing effect of acidosis declines in magnitude with increasing temperature [e.g. (Karatzaferi et al., 2008; Pate et al., 1995; Westerblad et al., 1997)]. Thus far it is not known if the uremic muscle's response to acidic conditions is similar to that of a control muscle and if any possible differences between uremic and control muscles observed in standard *in vitro* conditions would be evident at near physiological temperatures as well.

Overall, while intramuscular energetics disturbances, central activation failure, acidosis, as well as atrophy are implicated (Johansen et al., 2005) (Adams and Vaziri, 2006), the mechanisms underlying muscle dysfunction in CRI constitute a difficult aspect to precisely evaluate and are not

yet clear. Various interventions implemented so far, while beneficial (Gordon et al., 2007; Johansen et al., 2006; Sakkas et al., 2003b) have not fully corrected the functional deficits. Moreover, research so far has been mostly performed at the end-stage CKD and key issues related to CRI progression and contractile mechanisms are still unanswered.

To answer such questions we employed a surgically-induced animal model of CRI (Gotloib et al., 1982) to avoid confounding factors encountered in human patient studies (e.g. comorbidities, dialysis years, pharmaceuticals etc.) and we used permeabilized single muscle fibers (*aka* skinned fibers) to assess contractile machinery *per se* independently of metabolic and neural factors *in vitro* (Cooke and Bialek, 1979). This approach allows for the reliable study of sarcomeric function by assessing key mechanical parameters (such as maximal isometric force, velocity, etc.) under variable conditions of pH (Fitts et al., 1991) and temperature, using the temperature-jump method, with has high physiological relevance (Karatzaferi et al., 2008, 2004). Additionally, the single fiber approach allows one to better appraise the force generation capacity of individual fibers after adjusting for possible differences in muscle fiber size (by calculating specific force levels per fiber). Since the fast/glycolytic fibers, especially the most powerful ones expressing the IIX myosin heavy chain isoforms (Sakkas et al., 2003a; Sawant et al., 2011), are more prone to atrophy in renal disease, we examined the psoas muscle which is characterized by its homogeneity in fast IIX (IId) fibers (Aigner et al., 1993; Hämäläinen and Pette, 1993).

The aim of this study was to evaluate, for the first time, the effects of CRI on the contractile properties of isolated single muscle fibers, focusing on the function of contractile proteins *per se* independently of acute neurological and metabolic abnormalities. We examined, in a blind design, whether CRI affected the cell's ability to produce maximal isometric force and whether the contractile 'response' to acute acidosis was altered, at an earlier stage of renal insufficiency than available patient data. Moreover, considering the importance of temperature in translating our in vitro findings to in vivo function we examined contractile properties at a near physiological temperature.

Methods

Animal care and experimentation procedures were approved by the Ethics Committee of the University of Thessaly (decision 2-1/10-10-2012) and the Scientific Committee of the University Hospital of Larissa, Greece (decision 1/4-1-2012). Animals were under veterinary care, according to national and EU directives (Directive 2010/63/EU).

Animal model

New Zealand young adult white female rabbits (N=9) with an initial average body weight (BW) of 3200g were housed in a controlled environment with stable conditions (temperature 22–24°C, 12:12 h light-dark cycle) and acclimatized for 48h. Rabbits were fed *ad libitum* a special rabbit chow containing low levels of protein, potassium, calcium, phosphorus and sodium (Research Diets, Inc. USA) (reflecting dietary restrictions imposed on kidney disease patients) and water ad libitum.

After acclimatization, the induction of CRI was performed surgically (using a surgical protocol modified from Gotloib et al.) (Gotloib et al., 1982), under anesthesia and maintaining body temperature. Six animals (N=6) underwent removal of the left kidney after careful ligation of the left renal artery and vein, and partial nephrectomy (¾) of right kidney (UREM group). Three age–matched animals (N=3) underwent sham operation (CON group). In a previous pilot study (data not shown) sham-operated muscle contractility was comparable to non-procedure animals. Thus, in agreement with the principle of Reduction in Animal Research, sham-operated animals were used as controls.

Twelve weeks after surgery, animals were sacrificed by injection of sodium pentobarbital solution (50 mg/ml applied in a dosage of 100 mg/Kg BW) followed by bilateral thoracotomy. Immediately after cardiac arrest, blood samples were collected for subsequent determination of serum urea and creatinine using standard photometric protocols.

Muscle Samples

Psoas muscle samples from UREM and CON animals were fast excised and permeabilized as previously described (Karatzaferi et al., 2008). Briefly, thin bundles of rabbit psoas muscle (~2 mm diameter) were dissected, tied to wooden sticks using surgical thread, and placed in tubes containing the permeabilization buffer solution [120mM KAc, 50mM 3-(N-Morpholino) propanesulfonic acid (MOPS), 5mM $MgAc_2$, 4mM ethylene glycol tetraacetic acid (EGTA) and 50% glycerol (v/v); pH7] with the addition of 100μl/ 50 ml end volume, of a protease inhibitor cocktail (SIGMA P8340). Samples were gently shaken on a vibrating platform (Heidolph-Titramax 100) at 0°C. After 24 hours the buffer was replaced with fresh solution and samples were stored at -20^{o}C until mechanical assessments. All chemicals were purchased from Sigma-Aldrich.

Experimental setup for single fiber mechanics

Single fibers were dissected from the muscle bundle under a stereomicroscope on a cold stage and the fiber ends were attached between two tissue mounts of a customized micro-dynamometer (SI Heidelberg/WPI), connected to a force transducer (0.4 μN-4 N resolution) and a motor arm (used as a fixed end for isometric assessments). The fiber was then immersed in ‘baths’ each holding~200 μl

of experimental solution (see below). Data were continuously recorded and later exported for further analysis.

The micro-dynamometer consisted of a horizontally translocating platform with five independently temperature-controlled baths supported by a water circulator (Thermo Electron Haake WKL26). A He-Ne laser allowed for initial and end sarcomere length measurements as a quality check_and fibers were switched between baths in less than 250ms allowing for rapid temperature-jumps (t-jumps). As detailed elsewhere (Karatzaferi et al., 2004), the advantage of the t-jump is that by initially fully activating a fiber at low temperatures (here, 10^{o}C) the sarcomere arrangement was stabilized before being briefly exposed to a higher, near physiological temperature (here, 30^{o}C), generating maximal isometric force without damaging the sarcomere arrangement (for an indicative experiment see Figure 1). For more details in the experimental setup refer to the Supplement.

Experimental solutions

The basic rigor buffer contained: 120mM KAc, 5mM $MgAc_2$, 1mM EGTA and 50mM MOPS (pH7) or 50mM MES (pH6.2). Relaxing solution was achieved with the addition of 5mM ATP. Maximal calcium activation was achieved with the addition of 1.1mM $CaCl_2$. The ionic strength of the solutions was ~0.2M.

To exclude the possibility that the calcium concentration used to maximally activate control fibers might not be sufficient for uremic fibers, a small pilot study was first undertaken where fibers (data not shown) were blindly assessed in various calcium concentrations. It was concluded that the standard addition of 1.1mM $CaCl_2$ (pCa 4.4) provided maximal isometric force for both groups and was thereafter used.

Maximum Isometric Force measurements

All assessments and initial data reductions were done in a blind fashion. Each fiber was first immersed for 1 minute in a bath containing rigor solution (to wash out excess glycerol) and then transferred and equilibrated for 2 minutes in relaxing solution. Average diameter was determined for subsequent cross-sectional area (CSA) calculations assuming a cylindrical shape.

Maximum isometric force (Po) was first evaluated at standard resting conditions (pH7, 10^{o}C), at resting sarcomere lengths (2.2-2.4 μm). A number of fibers were subsequently activated at pH7 and 30^{o}C. A subset of fibers underwent an assessment at 10^{o}C, in both resting (pH7) and acidic (pH6.2) conditions while fewer fibers were also assessed at pH6.2, 30^{o}C. To avoid an order effect, fibers were randomly assigned to be first activated in pH7 and then in pH6.2 and *vice versa*. Lastly,

fibers were re-assessed in initial conditions (to fulfill criteria of stability, i.e. ≤10% initial force decline). For more details in maximum isometric force measurements see Supplement.

We also assessed velocity of contraction using the load-clamp method in a subset of fibers at 10 ^{o}C (see Supplement).

Statistical analysis

Force data distribution was tested using Kolmogorov-Smirnov test of normality. Due to the normal data distribution, statistical analysis was performed using parametric tests. Descriptive (Mean±SD) and Inferential (SEM and exact 95% CIs) statistics of absolute and specific forces, as well as percentage force values are reported. A General Linear Model (GLM) analysis was performed to examine main effects of independent variables and their interaction and also provided pairwise comparisons. To examine possible differences in the response to either temperature or pH changes, force change was calculated in percentages of initial standard conditions and the differences between groups were tested using t-test for independent samples. All statistical analyses were performed using a commercially available statistical package (SPSS 15.0). The significance level was set at $P<0.05$.

Results

Surgery procedures were well-tolerated and animals had a normal after-surgery recovery. Twelve weeks post-surgery, BW ranged between 1,970-4,585 and 3,500-4,965 gr for UREM and CON animals respectively ($P>0.05$), with higher serum creatinine (2.67±1.15 *vs* 1.38±0.09 mg/dl , $P<0.05$) and urea levels (67.33±32.02 *vs* 40.67±4.62 mg/dl, $P>0.05$) in UREM vs CON.

Cross –sectional area

Calculated cross sectional areas (CSAs) of UREM (n=240, 5,040±1,189 μm^2) fibers were significantly lower compared to CONs (n=142, 5,671±1,259 μm^2), $P<0.001$. Thus, the results were analyzed for both absolute and specific force values (i.e. force values corrected for fiber CSA to appraise force data independently of fiber atrophy).

Contractile properties in resting conditions (pH7)

Single psoas fibers (CON n=142, UREM n=240) were maximally calcium-activated at 10^{o}C, pH7 ('standard conditions'). Some fibers were also assessed at 30^{o}C, pH7 (CON n=41, UREM n=73) using the t-jump method. Descriptive and inferential statistics are presented in Table 1, Figure 2 and in the text.

Whether on absolute or specific force values, GLM analysis indicated a statistically significant main effect of *group* [$F(1,492)=83.6$, $P<0.001$ and $F(1,492)=33.1$, $P<0.001$ respectively], *temperature* [$F(1,492)=114.6$, $P<0.001$ and $F(1,492)=108.2$, $P<0.001$ respectively] as well as a significant interaction *group x temperature* [$F(1,492)=19.9$, $P<0.001$ and $F(1,492)=10.5$, $P=0.001$] for absolute and specific forces respectively.

The pairwise comparisons revealed consistent functional deficits in UREM fibers compared to CONs. At the standard conditions, absolute isometric force (Figure 2A) of UREM fibers was significantly lower (vs CON, $P<0.001$). After accounting for fiber CSA, UREM fibers were still found to produce lower specific forces (vs CON, $P<0.01$) (Figure 2B).

The t-jump caused an expected significant force rise in both groups ($P<0.001$). Still, UREM fibers produced significantly lower forces ($P<0.001$) than CON, at 30^{o}C, pH 7, in both absolute and specific forces, (Figure 2). After expressing the force response to the t-jump as a percentage of a fiber's own baseline force at pH7, 10^{o}C, UREM fibers appeared to gain more, as their average temperature-induced force increase was 2.2 fold that of CONs (+167±170% *vs* +76±42%, $P<0.005$) without however remedying the significant force disparity between groups.

Contractile properties in acidic conditions (pH6.2)

Lowering the pH, from 7 to 6.2, caused an expected significant force reduction in both groups ($P<0.005$, in 25 CON and 48 UREM fibers). Specifically, absolute (μN) and specific forces (mN/mm^2) at pH6.2, 10^{o}C, were for UREM fibers, 211±125 and 46±26 & for CON fibers, 316±116 and 55±20, respectively. Whether on absolute or specific force, GLM analysis indicated a statistically significant main effect of *pH* [$F(1,451)=22.9$, $P<0.001$, and $F(1,451)=21.7$, $P<0.001$ respectively], with the main effect of *group* being again significant [$F(1,451)=18.6$, $P<0.001$ and $F(1,451)=5.1$, $P<0.05$ respectively], but the interaction of *group x pH* was non-significant [$F(1,451)=0.003$, $P>0.05$, and $F(1,451)=0.028$, $P>0.05$ for absolute or specific force respectively].

The pairwise comparisons (using Bonferroni adjustments) showed that at 10^{o}C, pH6.2, the absolute isometric force (Figure 3A) of UREM fibers was significantly lower (vs CON, $P<0.05$) roughly by approx. -33%. UREM fibers still produced somewhat lower specific isometric forces, roughly by approx. -17% (Figure 3B) but non-significantly (vs CON, $P>0.05$).

After expressing the force response to the change of pH as a percentage of a fiber's own Po at standard conditions (pH7, 10^{o}C) the average % force decline due to the pH change tended to be larger for UREM fibers, albeit non-significantly (-48±14% *vs* -43±9%, $P=0.06$).

The temperature effect at pH6.2 was also assessed in a subset of fibers (UREM= 21, CON=13). Because it was not possible to lower the pH while at 30^{o}C, these data were not included

in the global statistical assessments mentioned above. The absolute (μN) and specific force (mN/mm^2) values at pH6.2, 30°C, were for UREM 550±248 and 114±52 & for CON fibers 700±238 and 117±33, respectively. The effect of the t-jump at pH6.2 was also expressed as percentage of force achieved at pH6.2, 10°C. The average temperature-induced force increase for UREM (+219±160%) and CON fibers (+143±62%) did not differ significantly (P>0.05).

Velocity of contraction

When examining the force-velocity relationship at 10°C (see Supplement), UREM muscle fibers (n=32) produced slower velocities compared to CON (n=15) in both resting and acidic conditions. As a result, Vmax of UREM fibers at either pH7 or pH6.2, was ~50% that of CON respectively.

Discussion

To the best of our knowledge this is the first study to examine the effect of renal insufficiency on the contractile properties of single skeletal muscle fibers, under resting and acidic (fatigue) conditions. We used methodology that is not acutely confounded by pervasive neural or metabolic abnormalities but instead focuses on sarcomeric function *per se*, independently of muscle atrophy. We found significantly impaired isometric force at the single fiber level, in muscle fibers from an animal model mimicking CRI, indicating a lower 'muscle quality'. This functional deficit was only partially explained by atrophy and was persistent under 'resting' and 'fatigue' conditions, under near physiological temperature conditions. We also observed evidence of slower velocities of contraction in UREM fibers (see the Supplement). We thus provide compelling evidence of CRI-induced effects on mechanical properties.

We observed significantly smaller CSAs in UREM psoas fibers compared to CON (by ~11%) in agreement to human studies. In end-stage disease, muscle atrophy is ~ 27% (Sakkas et al., 2003b) affecting mostly the fast type IIA and IIX muscle fibers (Sakkas et al., 2003a; Sawant et al., 2011). Recently Acevedo et al., (Acevedo et al., 2015) reported no evident atrophy in the tibialis anterior (a mixed fast hindlimb muscle) of surgically-induced uremic rats. In contrast, Organ et al., (Organ et al., 2016) reported atrophy in all fiber types of the extensor digitorum longus (another, mixed, fast muscle) of Cy/+ uremic rats. Together these and our results [in a larger species and a muscle expressing >95% IIX(IId) myosin, (Aigner et al., 1993; Hämäläinen and Pette, 1993)] could indicate a muscle type specificity of early atrophy in CRI. Our findings are consistent with human studies reporting muscle atrophy in advanced kidney patients (Johansen et al., 2003; Sakkas et al., 2003a; Sawant et al., 2011) and suggest that atrophy could appreciably affect fast muscles which are normally tasked to provide high levels of muscle power, earlier during the disease progress.

We found that at standard, resting conditions, UREM fibers produced significantly lower absolute and specific forces compared to CONs, by 25% and 14%, respectively at 10^{o}C, and by 40% and 28%, at 30^{o}C, respectively. Thus the degree of atrophy of uremic fibers only partially accounted for the force deficit. Organ et al., (Organ et al., 2016) attributed the reduced ankle dorsiflexion torque in Cy/+ uremic rats studied *in vivo* mostly to atrophy. They did not however assess single cell contractile properties and key study differences, such as in animal model and muscle group examined, could explain some discrepancy between our findings and previous results by Organ et al 2016 (Organ et al., 2016).

Muscle contraction is temperature sensitive (Ranatunga, 2010) and in agreement to previous reports (Coupland et al., 2001; Karatzaferi et al., 2008, 2004; Pate et al., 1995) both UREM and CON fibers substantially increased force generation in response to a t-jump from 10^{o}C to 30^{o}C. At pH7, force rise in CON fibers was ~76% in agreement to others using rabbit psoas skinned fibers (Coupland et al., 2001; Pate et al., 1995). Although the temperature-induced force increase was percent-wise higher in UREM, force at 30^{o}C remained significantly lower in UREM vs CON fibers. Thus the force deficit of the UREM fibers observed at standard *in vitro* conditions held also true at a near physiological temperature.

The deficit in UREM fibers' specific force could indicate a reduced capacity to generate force per myosin cross-bridge or a lower number of active cross-bridges (Fitts et al., 1991; Karatzaferi et al., 2004) and the slower velocities could indicate a slower cross-bridge cycle (see Supplement). Possible disturbances on force transmission across the sarcomeric arrangements may also be implicated, such as changes in viscoelastic properties [e.g. in human chronic heart failure (Miller et al., 2010)] or filament packing [e.g. hindlimb suspension (Riley, 2005)]. Other data from our group show increased protein carbonylation and other redox disturbances (Poulianiti et al., 2015), which could foreseeably cause structural modifications affecting the actomyosin interaction. Moreover, oxidative stress could also promote the formation of advanced glycation end-products, AGEs (Miyata et al., 1997), which in studies of reversible glycation (Ramamurthy et al., 2003, 2001), have been shown to cause glycation-related structural alterations' in myosin.

Fatigue intolerance in kidney disease has been associated with the rapid development of acidosis (Johansen et al., 2005; Moore et al., 1993). Acidosis alone or in combination with 'fatigue' metabolites, contributes to the inhibition of force generation at the cross-bridge level (Allen et al., 2008; Karatzaferi et al., 2008; Nelson and Fitts, 2014). Here, the drop of pH, reduced force by approx. 45% for both groups, in agreement to others (Cooke et al., 1988; Karatzaferi et al., 2003; Pate et al., 1995). The effect of acidosis may be less pronounced at near physiological temperatures in single fibers (Karatzaferi et al., 2008; Pate et al., 1995), but still significant. However, the

functional consequence of an acidosis-induced force reduction, coupled with a slower velocity of contraction (see Supplement), could be worse for uremic muscles *in vivo*. With uremic fibers exhibiting lower forces, and slower contractile velocities, even at resting conditions, one can fathom that in acidosis, even during low exercise intensities (Johansen et al., 2005) uremic muscles could be at a severe functional disadvantage.

Our study had many strengths but also some limitations. Despite implementing the same surgical approach, a large variability in uremic psoas fibers' contractile properties was observed. Also, it was not possible to evaluate all psoas fibers at 30^{o}C since many broke. Exactly due to this known sensitivity of skinned fibers we and others developed such t-jump protocols (Karatzaferi et al., 2008, 2004; Pate et al., 1995) while the majority of skinned fiber studies are performed at 10^{o}C-20^{o}C (Fitzsimons et al., 2001; Gilliver et al., 2010; Mollica et al., 2012; Murphy et al., 2004; Reggiani et al., 1997; Stienen et al., 1992; Sweeney et al., 1988). Also, UREM fibers protein extracts could not be resolved in SDS-PAGE (data not shown). Moreover, due to the blind design, a 'positive' bias was probably inadvertently introduced, with the 'better' UREM fibers being measured by each round of blind assessments: UREM fibers were in retrospect more difficult to dissect and handle. Furthermore, given some standard criteria for force data quality [e.g. (Karatzaferi et al., 2003; Liang et al., 2008)], it later transpired that relatively more uremic fibers' data were discarded than controls. Such discarded UREM fibers could be described as 'mussy' and 'sticky'. Based on the above, possible changes in passive elastic properties may warrant further study [e.g. changes in titin or nebulin may be implicated (Horowits et al., 1986)], as skeletal muscle viscoelastic properties changes, such as reported in heart failure patients (Miller et al., 2010; Toth et al., 2012) could be possible in our model. Advanced glycosylation has also been associated with glycation of type IV collagen of endothelial cells in ESRD (Thornalley and Rabbani, 2009) and further changes in overall muscle elastic properties cannot be excluded.

Main strengths of our study included: the use of the single fiber technique, which allows the assessment of sarcomeric function isolating factors such as muscle atrophy, energetics or excitation-contraction coupling issues; the blind design; the use of sham-operated controls. Moreover, our model developed CRI for 3 months, i.e. a sufficient period considering a rabbit's lifespan, making our results more relevant to the human chronic disease. Future studies should test the stretch-release force response and examine possible changes in sarcomeric viscoelastic properties, Moreover, possible post-translational modifications of key sarcomeric proteins, such as myosin, titin and nebulin, which according to our and other results may be affected in uremic myopathy, should be assessed and associated with further functional assessments.

In conclusion, experimentally-induced renal insufficiency led to significant functional impairments in single psoas fibers' mechanics, only partly explained by atrophy. Our observations, if verified in human tissue, can help explain key aspects of functional problems observed in patients.

Conflict of interest statement

The authors declare no conflict of interest.

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